THE OSMOTIC CONCENTRATION OF THE SAP OF THE LEAVES OF MANGROVE TREES.[1]

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I. PROBLEM AND METHODS.

The morasses of insular and continental coasts in the tropical zone of both eastern and western hemispheres are largely occupied by a vegetation consisting almost exclusively of a number of species of trees or shrubs collectively designated as mangroves.

These species, belonging to different genera and families, are characterized by striking morphological and biological features, for example, the prop or stilt roots of *Rhizophora*, the negatively geotropic roots, breathing roots or pneumatophores of *Avicennia*, and the remarkable vivipary and seedling dispersal of *Rhizophora*.

These various peculiarities have attracted the universal interest of biological travelers in the tropics.

The physiology of a group of organisms characterized by such morphological features and growing under environmental conditions so specialized presents a most interesting group of problems. When, however, one turns to the literature he is impressed by the fact that conclusions concerning the physiology of these plants are chiefly deductions from all too superficial field observations and from macroscopic and microscopic anatomical investigations. Physiological constants, properly so called, are practically wanting. Thus the recording of any quantitative data on the physiology of this ecologically fascinating group of plants is especially desirable. In the present paper we shall not summarize or comment upon the available literature, but shall limit ourselves to the presentation of the results of certain measurements carried out on the southern shore of the island of Jamaica

[1] Coöperative studies carried out by the Department of Experimental Evolution and the Department of Botanical Research of the Carnegie Institution of Washington.

in the winter of 1915 and in the Everglades region of southern Florida in the winter of 1916.

The specific problem attacked is that of the osmotic concentration of the tissue fluids.

It is quite natural that zoölogists should have led in the investigation of the relationship between the concentration of the tissue fluids and of that of the medium surrounding the organism. They have had at their disposal a wealth of forms exhibiting the widest possible range of organization, living in a medium which while showing a considerable range in concentration is nevertheless relatively constant over large areas, and opposes no obstacles to the easy extraction of samples of fluids (blood) for comparison with the medium. Among those who have contributed to the literature of this field may be mentioned Botazzi, Fredericq, Garry, Macallum, Green, Dakin, Mosso, Dekhuyzen, Scott, and others. The subject is discussed by R. Höber in his "Physikalische Chemie der Zelle und der Gewebe." Just as these pages were being prepared for the printer a summary by Scott ('16) of the data available for the depression of the freezing point of the blood of representatives of the various phyla of the animal kingdom, with the values obtained for the water in which the individuals were taken, appeared.

The problem of the osmotic concentration of the cell sap of the plant organism in relation to its medium has been rather extensively studied by a number of workers on the algæ and fungi. In recent years a number of investigations have also been carried out on the higher plants, both in the laboratory and in the field. These botanical studies need not be reviewed here.

It is perhaps clear that a study of arborescent plants growing in the saline substratum of the tropical coast has certain points of interest over that of the investigation of algæ or other submerged organisms. Not only is there the factor of the osmotic pull necessary in water ascent but there is the possibility of the increase in osmotic concentration of the foliar tissue fluids by the retention in solution (and hence in an osmotically active condition) of the salts absorbed from the concentrated substratum and left in the leaf tissue by the rapid transpiration incident to tropical temperature, insolation and air movements.

The purpose of this paper is to present the results of a series of determinations of the concentration of the leaf tissue fluids of the three genera, *Avicennia*, *Rhizophora* and *Laguncularia*.

This has been expressed in terms of molecules and ions as measured by the freezing point lowering Δ, corrected for undercooling (Harris and Gortner, '14), and in atmospheres pressure P as given in the paper just cited and in a supplementary table (Harris, '16) necessitated by the high concentrations found in the leaf tissues of the mangroves of the swamps on the southern shore of Jamaica and in the plants of the adjacent coastal desert (Shreve, '10). All determinations were based on sap extracted from tissue previously frozen (Gortner and Harris, '14), to increase its permeability, as emphasized by Dixon and Atkins ('13) and ourselves ('16). On approaching the freezing point or in passing it in undercooling, the sap of all three species generally shows a cloudiness or precipitation as noted by Gorke ('06). That this sensibly influences the freezing point lowering seems rather improbable, but we have not been able to give the subject adequate investigation.

Some difficulty was experienced with slight or heavy salt incrustations which sometimes occurred on the leaves, and are not easily removed without washing. To what extent this is of internal origin we are not able to assert. Probably it does not appreciably influence the constants upon which our discussions are based. Two determinations based on leaves of *Avicennia* from Port Henderson, Jamaica, which had particularly heavy incrustations, which were probably not thoroughly removed, gave:

Mar. 30, $\Delta = 5.86$, $P = 70.0$; April 2, $\Delta = 5.86$, $P = 70.0$.

II. Habitats from Which Collections Were Made.

The mangroves at Port Henderson form a dense thicket in a low area back of a gravelly, rather sterile beach which separates the swamp proper from the open sea. The vegetation consists of a dense growth of *Avicennia nitida* Jacq. with a few specimens of *Rhizophora mangle* L. and *Laguncularia racemosa* (L.) Gaertn. f. The pneumatophores of *Avicennia* thickly studded the water-covered soil or soft mud in most of the swamp.

The water here was not abnormally saline. One sample taken near the stilt roots of the collection of *Rhizophora* made January 22 gave $\Delta = 2.05$, $P = 24.7$. Another sample taken near the pneumatophores of *Avicennia* gave $\Delta = 1.96$, $P = 23.5$.

These determinations are in excellent agreement with those given by Garry ('15) for sea water from southern localities. Among his maximum values are $\Delta = 1.90$–1.93 for Pacific Grove, Cal., $\Delta = 2.04$ for Beaufort, N. C., and $\Delta = 2.24$ for Naples.

Back of the swamp which fringes a small bay with water too deep for the growth of either of the species, is a considerable area of almost sterile mud flat. Here the soil solution must become highly concentrated by evaporation from the superficial soil layers of the water left by occasional tidal overflow. We had no means of measuring this, but a sample of water from a slight depression, possibly diluted by a recent shower, gave when frozen $\Delta = 5.77$, $P = 68.9$. The mangroves occur only on the edges of these mud flats. Practically the only other species found here are the succulent-leaved halophytes, *Batis maritima* and *Sesuvium Portulacastrum*.

The determinations from subtropical Florida were based on plants growing on the mainland shore of Biscayne Bay at Miami and Cocoanut Grove and on the Everglades or Front Prairie, as some term it, south of Florida City.

A single sample of Biscayne water taken near the shore at Cocoanut Grove gave $\Delta = 1.45$, $P = 17.4$. This determination is distinctly lower than those cited for the seacoast localities, and more nearly comparable with the $\Delta = 1.09$ for Kiel Harbor, $\Delta = 1.30$ for the open Baltic sea, or $\Delta = 1.66$ as determined in the Kattegat by Dakin and cited by Garry (*loc. cit.*).

The dwarfed mangroves growing on the Everglades near Florida City are in a practically non-saline substratum. A sample of water from a ditch froze at $-.009°$, a value in good agreement with that for bog and pond water as studied by Livingston ('04) and Transeau ('16).

Scott ('16) classifies water with a depression of 0.03° as fresh.

III. Presentation of Constants.

A. Avicennia nitida Jacq.

The leaves of trees of *Avicennia nitida* from the Port Henderson swamp proper gave:

Jan. 20, $\Delta = 4.26$, $P = 51.1$
Jan. 22, $\Delta = 3.29$, $P = 39.4$
Jan. 22, $\Delta = 3.56$, $P = 42.7$
Mar. 26, $\Delta = 3.47$, $P = 41.5$
Mar. 30, $\Delta = 3.60$, $P = 43.2$
April 2, $\Delta = 3.84$, $P = 46.0$

Average, $\overline{\Delta} = 3.67$, $\overline{P} = 43.98$

Plants from the edges of the sterile mud flats gave the following values:

Jan. 20, $\Delta = 3.67$, $P = 43.9$
Jan. 25, $\Delta = 4.55$, $P = 54.4$
Mar. 26, $\Delta = 3.90$, $P = 46.7$
April 2, $\Delta = 4.29$, $P = 51.3$

Average, $\overline{\Delta} = 4.10$, $\overline{P} = 49.08$

Thus on the southern coast of Jamaica *Avicennia nitida* is characterized by leaf sap showing a concentration of about 45 to 50 atmospheres. In view of the considerable variation in the individual determinations it is impossible to assert that the concentration in the plants growing on the edge of the sterile mud flats is *significantly* higher than that of those occurring in the swamp proper.

For the most part, the leaves taken were fully mature, but on Jan. 20th, it was possible to secure some new-growth leaves in the collection from the mud flats. These gave $\Delta = 3.56$, $P = 42.6$ as compared with $\Delta = 3.67$, $P = 43.9$ for the old leaves. Thus the old and the young organs show about the same concentration of tissue fluids.

Two collections of the leaves of seedlings, about 1–2 dm. in height, growing in mud or shallow water in the rather dense shade of the trees gave:

Jan. 20, $\Delta = 4.47$, $P = 53.5$
Jan. 22, $\Delta = 4.02$, $P = 48.1$

Note that these values are actually slightly in excess of those determined from the leaves of the trees collected on the same dates.

A tree of *A. nitida* about 16 cm. in diameter on the mainland shore of Biscayne Bay at Miami gave:

Feb. 24, $\Delta = 2.76$, $P = 33.1$

This constant is distinctly lower than any of those determined at Port Henderson, and indicates that the latter values are influenced by the growth of the plants under the general environmental conditions which have resulted in the development in this region of the coastal desert flora (Harris and Lawrence, '17).

B. Rhizophora mangle L.

Rhizophora was not abundant on the Jamaican coast where our collections were made. The shrubs occurred only in the swamp. The constants determined were:

Jan. 22, $\Delta = 2.47$, $P = 29.6$
Jan. 25, $\Delta = 2.57$, $P = 30.9$
Mar. 26, $\Delta = 2.43$, $P = 29.2$
Mar. 30, $\Delta = 2.53$, $P = 30.4$
April 2, $\Delta = 2.49$, $P = 29.9$

Average, $\bar{\Delta} = 2.50$, $\bar{P} = 30.0$

Small trees of *R. mangle* on the mainland shore of Biscayne Bay yielded leaves which gave the following concentrations:

Feb. 8, $\Delta = 2.20$, $P = 26.4$
Feb. 17, $\Delta = 2.24$, $P = 26.9$
Feb. 24, $\Delta = 1.95$, $P = 23.4$

Average, $\bar{\Delta} = 2.13$, $\bar{P} = 25.57$

On the prairie below Florida City *Rhizophora* grows in a substratum where the amount of salt in the soil must be insignificant. Here the constants were found to be:

Feb. 18, $\Delta = 1.85$, $P = 22.2$
Feb. 29, $\Delta = 1.84$, $P = 22.1$
Feb. 29, $\Delta = 1.91$, $P = 22.6$

Average, $\bar{\Delta} = 1.87$, $\bar{P} = 22.30$

The reader will have noticed three salient results in these constants. First, the concentrations in *Avicennia* are distinctly higher than in *Rhizophora*. Second, that the Florida collections give distinctly lower constants than do those made in Jamaica. Third, that there is a distinct reduction in the osmotic concentration as the Florida plants leave the shores of the bay and penetrate into the non-saline substratum of the Everglades region.

C. *Laguncularia racemosa* Gaertn. f.

At Port Henderson, *Laguncularia racemosa* occurs both in the swamp and on the relatively sterile mud flats.

In the swamp the trees gave:

Jan. 22, $\Delta = 2.07$, $P = 24.9$
Jan. 22, $\Delta = 2.05$, $P = 24.6$
Mar. 26, $\Delta = 2.13$, $P = 25.6$
Mar. 30, $\Delta = 2.14$, $P = 25.7$
Average, $\bar{\Delta} = 2.10$, $\bar{P} = 25.20$

On the relatively sterile mud flats the concentrations were:

Jan. 20, $\Delta = 2.90$, $P = 34.8$
Jan. 25, $\Delta = 2.90$, $P = 34.7$
Mar. 26, $\Delta = 2.79$, $P = 33.5$
Mar. 30, $\Delta = 2.69$, $P = 32.3$
April 2, $\Delta = 2.75$, $P = 33.1$
Average, $\bar{\Delta} = 2.81$, $\bar{P} = 33.68$

While it was not possible to assert that the osmotic concentration of the leaf sap of *Avicennia* was significantly higher when growing on the sterile mud flats, there can be no reasonable question of the relative values of the constants in the case of *Laguncularia*. The five individual values from the flats are without exception higher than the four available from the swamp.

Two large trees of *L. racemosa* on the mainland shore of Biscayne Bay gave:

Miami, Feb. 17, $\Delta = 2.11$, $P = 25.3$
Cocoanut Grove, Feb. 26, $\Delta = 2.24$, $P = 26.9$

Young trees about 1½ m. high gave:

Miami, Feb. 24, $\Delta = 1.81$, $P = 21.7$
Cocoanut Grove, Feb. 26, $\Delta = 1.73$, $P = 20.8$

That the sap of *L. racemosa* is profoundly influenced by its substratum is splendidly shown by a determination based on trees growing in practically fresh water on the south shore of Jamaica. An irrigation canal passes through the coastal desert. Trees[1] growing in the marsh beside it gave:

Jan. 30, $\Delta = 1.64$, $P = 19.7$

IV. Recapitulation.

The present paper is a contribution to the problem—hitherto practically untouched by quantitative methods—of the physiology of mangrove vegetation. Specifically it deals with the osmotic concentration or osmotic pressure of the leaf tissue fluids.

Three species, *Avicennia nitida* of the Avicenniaceæ, *Rhizophora mangle* of the Rhizophoraceæ, and *Laguncularia racemosa* of the Terminaliaceæ have been examined on the southern shore of the island of Jamaica and in southern Florida. Determinations of freezing point lowering of sap extracted from plants growing in habitats ranging from a substratum saturated with fresh water to the margins of highly saline and practically sterile mud flats have been secured.

The concentration of the tissue fluids is relatively high throughout. The minimum concentrations are about 20–22 atmospheres for *Laguncularia* and *Rhizophora* growing under practically fresh-water influence and 33 atmospheres for *Avicennia* on the shore of Biscayne Bay. The values may range widely in response to local conditions. Thus on the southern coast of Jamaica where the plants are growing under the influence of the edaphic and meterological conditions which on higher levels have resulted in a splendidly developed desert vegetation, *Avicennia* shows trustworthy measurements of concentrations up to fifty atmospheres in leaves in a fairly healthy condition. It is quite probable that somewhat higher values will be found.

[1] Determinations on three more or less herbaceous forms were secured for comparison

Ammania latifolia L.	April 2,	$\Delta = 1.60$, $P = 19.3$
Pluchea purpurascens (Sw.) D.C.	April 4,	$\Delta = 1.21$, $P = 14.5$
	March 30,	$\Delta = 1.25$, $P = 15.0$
Verbesina alba L. (*Eclipta alba* (L.) Hassk.)	April 12,	$\Delta = 1.60$, $P = 19.2$

Rhizophora mangle shows concentrations of about 25 atmospheres on the mainland shore of Biscayne Bay and 30 atmospheres in the swamp at Port Henderson, Jamaica, as compared with slightly over 22 atmospheres under the practically fresh-water influence of the Everglades. *Laguncularia racemosa* shows a concentration of about 20 atmospheres under nearly fresh-water influence at Port Henderson, from 20 to 25 atmospheres on the shore of Biscayne Bay, about 25 atmospheres in the mangrove swamp and about 34 atmospheres on the edges of the sterile mud flats near Port Henderson.

Apparently *Avicennia* is capable of developing a much higher sap concentration than either of the other species investigated. Thus on the shore of Biscayne Bay it showed a concentration of about 33 atmospheres, whereas the maximum values for *Rhizophora* and *Laguncularia* were about 27 atmospheres. In the swamp at Port Henderson the leaves of trees of *Avicennia* gave a freezing point lowering indicating a concentration of about 44 atmospheres as compared with 30 atmospheres for *Rhizophora* and about 25 atmospheres for *Laguncularia*. Even the leaves of seedling plants of *Avicennia* exhibited a concentration of about 50 atmospheres as compared with the values just cited for adult leaves of *Rhizophora* and *Laguncularia*.

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